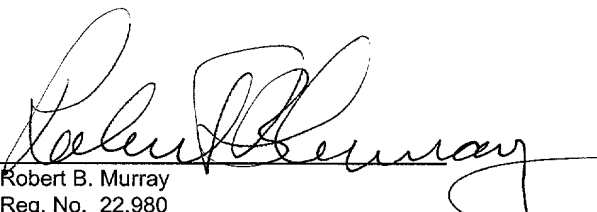


FORM PTO-1390 (REV 5-93)		U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE		ATTORNEY DOCKET NO. P100564-00002	
TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371				DATE: March 23, 2000	
				U.S. APPLN. NO. (IF KNOWN, SEE 37 CFR 1.5) 09/508510	
INTERNATIONAL APPLICATION NO. PCT/EP98/06065		INTERNATIONAL FILING DATE 23 September 1998		PRIORITY DATE CLAIMED 23 September 1997	
TITLE OF INVENTION: LIQUID INTERFERON- β FORMULATIONS					
APPLICANT(S) FOR DO/EO/US: Michael TSCHÖPE, Thomas SIKLOSI, Peter SHROEDER, Hans HOFER					
<p>1. <input checked="" type="checkbox"/> This is a FIRST submission of items concerning a filing under 35 U.S.C. 371. (THE BASIC FILING FEE IS ATTACHED)</p> <p>2. <input type="checkbox"/> This is a SECOND or SUBSEQUENT submission of items concerning a filing under 35 U.S.C. 371.</p> <p>3. <input checked="" type="checkbox"/> This express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and PCT articles 22 and 39(1).</p> <p>4. <input checked="" type="checkbox"/> A proper demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.</p> <p>5. <input checked="" type="checkbox"/> A copy of the International Application as filed (35 U.S.C. 371(c)(2))</p> <p>a. <input checked="" type="checkbox"/> is transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input checked="" type="checkbox"/> has been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> is not required, as the application was filed in the United States Receiving Office (RO/US)</p> <p>6. <input checked="" type="checkbox"/> A translation of the International Application into English (35 U.S.C. 371(c)(2)).</p> <p>7. <input checked="" type="checkbox"/> Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))</p> <p>a. <input checked="" type="checkbox"/> are transmitted herewith (required only if not transmitted by the International Bureau).</p> <p>b. <input type="checkbox"/> have been transmitted by the International Bureau.</p> <p>c. <input type="checkbox"/> have not been made; however, the time limit for making such amendments has NOT expired.</p> <p>d. <input type="checkbox"/> have not been made and will not be made.</p> <p>8. <input checked="" type="checkbox"/> A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).</p> <p>9. <input type="checkbox"/> An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).</p> <p>10. <input type="checkbox"/> A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).</p> <p>Items 11. to 16. below concern other document(s) or information included:</p> <p>11. <input checked="" type="checkbox"/> An Information Disclosure Statement under 37 CFR 1.97 and 1.98.</p> <p>12. <input type="checkbox"/> An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.</p> <p>13. <input type="checkbox"/> A FIRST preliminary amendment. <input type="checkbox"/> A SECOND or SUBSEQUENT preliminary amendment.</p> <p>14. <input type="checkbox"/> A substitute specification.</p> <p>15. <input type="checkbox"/> A change of power of attorney and/or address letter.</p> <p>16. <input checked="" type="checkbox"/> Other items or information: PCT/IB/306 (2), PCT/R0/101, PCT/IPEA/409, PCT/ISA/210 CHECK NO. 288153</p>					

422 Rec'd PCT/PTO 23 MAR 2000

U.S. APPLN. NO. (IF KNOWN, SEE 37 C.F.R. 1.50) <div style="font-size: 24pt; font-weight: bold; margin-top: 10px;">09/508510</div>		INTERNATIONAL APPLICATION NO. PCT/EP98/06065		ATTORNEY DOCKET NO. P100564-00002 DATE: March 23, 2000	
17. <u>XX</u> The following fees are submitted: Basic National Fee (37 CFR 1.492(a)(1)-(5): Search Report has been prepared by the EPO or JPO.....\$840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482)...\$670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)).....\$760.00 Neither international preliminary examination fee (37 CFR 1.482) or international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....\$970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4)\$ 96.00				CALCULATIONS PTO USE ONLY <hr/>	
ENTER APPROPRIATE BASIC FEE AMOUNT =				\$840	
Surcharge of \$130.00 for furnishing the oath or declaration later than _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				\$00	
Claims	Number Filed	Number Extra	Rate		
Total Claims	- 20 =		X \$ 18.00	\$00	
Independent Claims	- 3 =		X \$ 78.00	\$00	
Multiple dependent claim(s) (if applicable)			+ \$260.00	\$00	
TOTAL OF ABOVE CALCULATIONS =				\$840	
Reduction by 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				\$00	
SUBTOTAL =				\$840	
Processing fee of \$130.00 for furnishing the English translation later the _ 20 _ 30 months from the earliest claimed priority date (37 CFR 1.492(f)).				\$00	
TOTAL NATIONAL FEE =				\$840	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). \$40.00 per property				\$00	
TOTAL FEES ENCLOSED =				\$840	
				Amount to be refunded	\$
				Charged	\$
a. <u>XX</u> A check in the amount of \$840 to cover the above fees is enclosed. b. _ Please charge my Deposit Account No. <u>01-2300</u> in the amount of \$_____ to cover the above fees. A duplicate copy of this sheet is enclosed. c. _ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. <u>01-2300</u> . NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status. SEND ALL CORRESPONDENCE TO: Arent Fox Kintner Plotkin & Kahn PLLC 1050 Connecticut Avenue, N.W., Suite 600 Washington, D.C. 20036 Telephone No. (202) 857-6000					
 Robert B. Murray Reg. No. 22,980					

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Michael TSCHÖPE et al

Serial No.: 09/508,510

Filed: March 23, 2000

For: LIQUID INTERFERON- β FORMULATIONS

PRELIMINARY AMENDMENT

Commissioner of Patents
Washington, D.C. 20231

May 26, 2000

Sir:

Prior to calculation of the filing fee and prior to the examination of this application,
please amend the above-identified application as follows:

IN THE CLAIMS:

Claim 6, line 1, delete "any of Claims 1 to 5" and insert therefor --claim 1--.

Claim 7, line 1, delete "any of Claims 1 to 6" and insert therefor --claim 1--.

Claim 9, line 1, delete "any of Claims 1 and 3 to 8" and insert therefor --claim 1--.

Claim 11, line 1, delete "any of Claims 1 to 10" and insert therefor --claim 1--.

Claim 12, line 1, delete "any of Claims 1 to 11" and insert therefor --claim 1--.

Claim 13, line 1, delete "any of Claims 1 to 12" and insert therefor --claim 1--.

Claim 14, line 1, delete "any of Claims 1 to 13" and insert therefor --claim 1--.

Claim 15, line 1, delete "any of Claims 1, 2 and 4 to 14" and insert therefor --claim
1--.

Claim 16, line 1, delete "or 15".

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Claim 18, line 1, delete "any of Claims 1 to 17" and insert therefor --claim 1--.

Claim 19, line 1, delete "any of Claims 1 to 18" and insert therefor --claim 1--.

Claim 20, line 1, delete "any of Claims 1 to 19" and insert therefor --claim 1--.

Claim 21, lines 3 and 4, delete "any of Claims 1 to 20" and insert therefor --claim 1--.

Claim 23, line 2, delete "or 22".

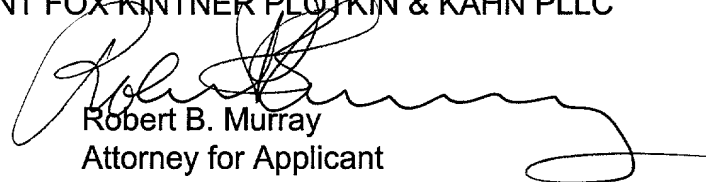
Cancel claim 24 without prejudice.

REMARKS

The above amendment to the claims has been made to correct the multiple dependency of the claims and to put the application in better condition for examination.

In the event that any fees are due in connection with this paper, please charge our Deposit Account No. 01-2300.

Respectfully submitted,
ARENT FOX-KINTNER PLOTKIN & KAHN PLLC


Robert B. Murray
Attorney for Applicant
Reg. No. 22,980

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RBM/cb

Liquid interferon- β formulations

Description

5 The present invention relates to liquid formulations of human interferon- β . The formulations are characterized in that they have a pH in the weakly acidic to neutral range between 5 and 8 and that the interferon- β is highly stable in solution while retaining the molecular
10 integrity.

Naturally occurring interferons are species-specific proteins, in some cases glycoproteins, which are produced by various cells of the body after induction
15 with viruses, double-stranded RNA, other polynucleotides and antigens. Interferons exhibit a large number of biological activities such as, for example, antiviral, antiproliferative and immunomodulatory properties. At least 3 different types of human
20 interferons have been identified; they are produced by leucocytes, lymphocytes, fibroblasts and cells of the immune system and termed α -, β - and γ -interferons. Individual types of interferons can furthermore be divided into a large number of subtypes.

25 Native, human interferon- β can be prepared commercially by superinduction of human fibroblast cell cultures with poly-IC followed by isolation and purification of the interferon- β by chromatographic and electrophoretic
30 techniques. Proteins or polypeptides which exhibit properties similar to those of natural interferon- β can also be prepared by recombinant DNA technologies (EP-A-0 028 033; EP-A-0 041 313; EP-A-0 070 906; EP-A-0 287 075; Chernajovsky et al. (1984) DNA 3,
35 297-308; McCormick et al. (1984) Mol. Cell. Biol. 4. 166-172). Recombinant human interferon- β can be produced both in eukaryotic cells (for example CHO cells) and by prokaryotic cells (for example E. coli). The interferons in question are termed interferon- β -1a

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and interferon- β -1b respectively. In contrast to interferon- β -1b, interferon- β -1a is glycosylated (Goodkin (1994) Lancet 344, 1057-1060).

5 A prerequisite for the therapeutic use of interferon- β is that it is pharmaceutically formulated so that the protein is storage-stable over a prolonged period while retaining the molecular integrity. Interferon- β is unstable and subject to various degradation reactions.
10 These include, in particular, the cleavage of peptide bonds, deamidation, oxidation of the methionin to methionin sulphide, disulphide exchange, and changes in the sugar side chain which even include deglycosylation.

15 Owing to the therapeutical benefit of interferons, a series of formulations have been developed in recent years; however, all of them exhibit certain disadvantages. US Patent No. 4,647,454 (Inter-Yeda
20 Ltd.) describes a formulation of fibroblast interferon- β which can be stabilized by addition of polyvinylpyrrolidone (PVP) in the highly acidic range (pH 3.5). Other preferred auxiliaries are mannitol, human serum albumin and acetate buffers. The
25 formulation is freeze-dried and stored at 4°C.

The Japanese Patent Specification 59 181 224 (Sumitomo Chemical Co.) describes an aqueous solution of interferons in which polar amino acids such as
30 arginine, asparagine, glutamic acid, glutamine, histidine, lysine, serine and threonine and their sodium salts together with human serum albumin are employed for stabilizing the interferons.

35 The international Patent Application WO 95/31213 (Applied Research Systems ARS Holding) describes a liquid formulation for interferon- β which is stabilized by addition of a polyol, preferably mannitol, and a non-reducing sugar or an amino acid. The formulation

furthermore comprises a buffer (acetate buffer pH 3.0 to 4.0) and human serum albumin. While formulas with a pH of between 5 and 6 showed an immediate loss in biological activity, the formulas preferred in the patent specification are sufficiently stable at pH values of 3.0 and 4.0. Moreover, the statement regarding stability only refers to the biological activity of the formulation, but not to the molecular integrity of the active ingredient.

10

The European Patent Application EP 0 215 658 (Cetus Corp.) describes a formulation for recombinant interferon- β in which the bioactive compound is dissolved in an aqueous medium at a pH of between 2 and 4 with addition of stabilizers such as human serum albumin or human plasma protein fractions and, if appropriate, dextrose. A further patent application of Cetus Corp. (WO 89/05 158) describes a formulation for interferon- β where either glycerin or polyethylene glycopolymers with a mean molecular weight of between 190 to [sic] 1600 daltons are employed as stabilizers at a pH of between 2 and 4. Suitable buffer components which are mentioned are glycine, phosphoric acid and citric acid.

25

The European Patent Application EP 0 217 645 (Cetus Corp.) describes pharmaceutical preparations with IL-2 or interferon- β which are dissolved in an excipient medium at pH 7 to 8 and stabilized with addition of sodium laurate as surfactant. In addition, SDS is also required as further ionic surfactant in order to stabilize these preparations.

The European Patent EP 0 270 799 (Cetus Oncology Corp.) describes a formulation for unglycosylated recombinant interferon- β in an inert water-based excipient medium which comprises non-ionic polymeric detergents as stabilizer.

The European Patent Application EP 0 529 300 (Rentschler Biotechnologie GmbH) describes liquid interferon- β formulations which comprise a concentration of 30 or 70 MU/ml recombinant IFN- β , sodium chloride and imidazole buffer or sodium phosphate buffer and have a pH of 7.5 (Example 3). These formulations are stable with regard to their biological activity for 4 weeks at a storage temperature of 25°C. However, the disadvantage of these compositions is that the concentration of interferon- β used (≥ 30 MU/ml) is too high for practical applications. Moreover, there is no mention in EP-A-0 529 300 of a reduction in the stability of liquid interferon- β formulations by addition of human serum albumin. In contrast, the addition of human serum albumin is stated as being preferred.

In addition to formulations for interferon- β , there are also described pharmaceutical dosage forms with interferon- α . The European Patent Specification 0 082 481 (Schering Corp.) discloses an aqueous formulation intended for freeze-drying which comprises human serum albumin, in addition to a phosphate buffer and glycine. Alanine is mentioned as further optional constituent. After reconstitution, the pH of the solution is between 7.0 and 7.4. A further patent application of Schering Corp. (WO 96/11018) discloses stable aqueous solutions in interferon- α which comprise chelating agents (NaEDTA or citric acid), a surfactant (Polysorbat 80), an isotonizing agent (sodium chloride) and suitable preservatives such as methylparaben, propylparaben, m-cresol or phenol, at a pH of between 4.5 and 7.1. With regard to the biological activity (standard method of inhibiting the cytopathic effect (CPE) of a virus as described by W.P. Protzman in J. Clinical Microbiology, 1985, 22, pp. 596-599), the aqueous formulations disclosed prove to be stable for 6 months at 25°C (biological activity $>90\%$ of the initial activity). However, a determination of the

protein content by HPLC carried out in parallel already shows losses in content of between 20.2 (Table 3) or 32.5% (Table 4) after 6 months at 25°C.

- 5 EP-A-0 736 303 (Hoffmann-LaRoche AG) discloses aqueous interferon- α compositions which, in addition to an interferon- α , comprise a non-ionic detergent, a buffer for setting the pH range between 4.5 and 5.5, benzyl alcohol and, if appropriate, isotonicizing agents. A
10 determination by HPLC identifies a residual content of 84.5% after storage for three months at 25°C and a starting concentration of 18 MU interferon- α 2a, while this value drops to 62.8% when the stabilizer benzyl alcohol is omitted.
- 15 EP-A-0 641 567 (Ciba Geigy AG) describes pharmaceutical compositions which comprise hybrid interferon- α and, as stabilizer, a buffer with a pH of between 3.0 and 5.0.
- 20 US Patent 5,358,708 (Schering Corp.) describes aqueous formulations of interferon- α which comprise methionine, histidine or mixtures of these as stabilizer. After storage of an interferon- α solution at 40°C for two weeks, it is found that the active ingredient content
25 has decreased by 20%.

The abovementioned formulations for interferons have shortcomings from the present-day view since, for example, an addition of human serum albumin for
30 stabilizing proteins should be dispensed with, owing to the higher demands for safety from virus contamination by blood donors. Moreover, a number of the above-described formulations require the addition of amino acids and/or freeze-drying. However, freeze-dried
35 products are complicated to produce and, accordingly, expensive and require an additional pass owing to the necessity of reconstitution, and this additional pass is frequently very difficult to perform, in particular for patients with a limited power of movement. A series

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of formulas have unphysiological pH values of below 5.0. While such values are not entirely unusual (see also S. Sweetana and N.J. Aders, Journal of Pharmaceutical Sciences and Technology, 1996, 50: 330-342), painful irritation must be expected in the case of intramuscular or subcutaneous application. While according to Sweetana and Akers the use of surfactants such as Polysorbate 80 is admissible, a series of side effects have been described, in particular in new-born and older children, which make the use of such auxiliaries questionable. A review of the toxicity of surfactants can be found in Attwood and Florence (Surfactant Systems, their Chemistry, Pharmacy and Biology, Chapman and Hall; London, 1983). The pharmacology of Polysorbate 80 is reviewed by R.K. Varma et al. (Arzneim.-Forsch./Drug Res. 35, 1985, 804-808).

On the basis of the abovementioned disadvantages, an optimal formulation for interferon- β should combine the following properties:

- retaining the biological activity over the storage period,
- retaining the molecular integrity of the active ingredient molecule over the storage period,
- liquid formulation, no expensive freeze-drying and no additional reconstitution,
- no risky auxiliaries such as human serum albumin or surfactants (detergents),
- pH in the neutral to weakly acidic range.

All requirements are met by the invention, which is described in greater detail in the section which follows.

Surprisingly, a composition of a formula has been found which ensures the molecular integrity of interferon- β in liquid form over a prolonged period in a physiological pH range of between 5 and 8, preferably

between over 5.5 and 8, without having to resort to the auxiliaries of the prior art, which are known as being disadvantageous.

5 A first aspect of the present invention is therefore a liquid pharmaceutical formulation which comprises human interferon- β as active ingredient in a concentration of up to 25 MU/ml and a buffer for setting a pH of between 5 and 8, preferably between over 5.5 and 8, is free
10 from human serum albumin and shows a long-term stability of the biological activity (in vitro) of at least 80% of the initial activity after storage for 3 months at 25°C.

15 A further aspect of the invention is a liquid pharmaceutical formulation which comprises human interferon- β as active ingredient and a buffer for setting a pH of between 6 and 7.2, is free from human serum albumin and shows a long-term stability of the
20 biological activity (in vitro) of at least 80% of the initial activity after storage for 3 months at 25°C.

Yet a further aspect of the invention is a liquid pharmaceutical formulation which comprises human IFN- β
25 as active ingredient, a buffer for setting a pH of between 5 and 8, preferably between over 5.5 and 8, and one or more amino acids and shows a long-term stability of the biological activity (in vitro) of at least 80% of the initial activity after storage for 3 months at
30 25°C.

The long-term stability of liquid pharmaceutical formulations was measured at 25°C. The temperature of 25°C was chosen, on the one hand, to cause accelerated
35 degradation reactions, but, on the other hand, to avoid artefacts caused by unduly high temperatures. Suitable analytical methods for determining the stability of interferon- β can be found in the reviews by J. Geigert (J. Parent. Sci. Technol. 43 (1989), 220-224) or

M.C. Manning, K. Patel and R.T. Borchardt (Pharm. Res. 6 (1989), 903-918).

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The biological activity after the storage period chosen
5 in each case was measured by the standard method of
inhibiting the cytopathic effect of a virus. A detailed
description of the test method used can be found in
Stewart, W.E. II (1981): The Interferon System (Second,
enlarged Edition), Springer-Verlag: Vienna, New York;
10 Grossberg, S.E. et al. (1984), Assay of Interferons.
In: Came, P.E., Carter W.A (eds) Interferons and their
Applications, Springer-Verlag: Berlin, Heidelberg, New
York, Tokyo, pp. 23-43. After storage for three months
at 25°C, a formulation according to the invention
15 exhibits a biological activity of at least 80%,
preferably of at least 85%, and especially preferably
of at least 90%, of the initial activity.

After storage for six months at 25°C, a formulation
20 according to the invention preferably has a biological
activity of at least 80%, and preferably of at least
85%, of the initial activity.

Even when stored at higher temperatures, for example
25 37°C, the formulations according to the invention
exhibit a surprisingly high long-term stability of the
biological activity. For example after storage for one
month at 37°C, a biological activity of at least 70%,
and preferably of at least 80%, of the initial activity
30 is found.

The liquid pharmaceutical formulations according to the
invention are preferably free from human serum albumin
and especially preferably - apart from the active
35 ingredient - free from human or animal polypeptides, in
particular serum proteins. It is furthermore preferred
for the liquid pharmaceutical formulation according to
the invention to be free from surfactants, in
particular free from ionic detergents and/or non-ionic

surfactants.

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The formulations according to the invention comprise, as active ingredient, an interferon- β , that is to say a polypeptide which exhibits biological and/or immunological properties of natural human interferon- β and which may be a naturally occurring or recombinant interferon- β . The formulation preferably comprises a glycosylated interferon- β , especially preferably a recombinant interferon- β from CHO cells. Interferon- β species which are most preferably used are those which can be obtained from the cell line BIC 8622 (ECACC 87 04 03 01) and which are described, for example, in EP-B-0 287 075 and EP-A-0 529 300.

15 Preferably, the active ingredient is present in the formulations according to the invention in a concentration of up to 25 MU/ml. However, a dosage in the range of 1 to 25 MU/ml is preferred, in the range of 3 to 20 MU/ml especially preferred and in the range of 3 to 10 MU/ml most preferred. These dosage ranges allow an immediate use without further dilution in conjunction with a particularly good stability at an elevated temperature.

25 A further preferred feature of the liquid pharmaceutical formulation according to the invention is that it exhibits a chemical integrity after storage for 3 months, and preferably 6 months, at 25°C, i.e. that it is stable to peptide cleavage, oxidation and deglycosylation. The chemical integrity is measured by peptide mapping, Western blot and glycosylation analysis. Chemically stable for the purposes of the present invention are compositions in which the interferon- β after formulation retains at least 85%, preferably at least 90%, of the chemical integrity at the storage conditions chosen.

A further preferred feature of the liquid

pharmaceutical formulations according to the invention is a physical integrity after storage for 3 months, and preferably 6 months, at 25°C. The physical integrity is in this case measured by measuring the transmission at
5 420 nm and by visually observing the solutions. Physically stable are those solutions whose transmission is over 90%, preferably over 93%, at the storage conditions chosen, and where no turbidity can be determined upon visual observation.

10

The present invention surprisingly allows liquid formulations of interferon- β to be provided which are biologically, chemically and physically stable over a prolonged period and free from undesired constituents
15 such as, for example, human serum albumin or surfactants. In addition to the active ingredient, the formulations according to the invention comprise a buffer which is preferably present in a concentration of 10 mmol/l to 1 mol/l, especially preferably in a
20 concentration of 20 mmol/l to 200 mmol/l, for example approximately 50 mmol/l to 100 mmol/l, and which serves to maintain the pH of the formulation in the range of 5 to 8, preferably above 5.5 to 8, more preferably between 6 and 7.4. A pH range between 6 and 7.2 is
25 especially preferred, and a pH range between 6.2 and 6.8 most preferred, since a particularly high stability while retaining the molecular integrity is achieved here. The buffer is selected from amongst pharmaceutically acceptable buffers, for example
30 borate, succinate, L-malate, TRIS, salicylate, glycylglycine, triethanolamine, isocitrate, maleate, phosphate, citrate and acetate buffer, or mixtures of these. Phosphate, citrate and acetate buffer or mixtures of these are preferably used, especially
35 preferably phosphate/citrate buffers.

In addition to the active ingredient and the buffer, the formulation according to the invention can comprise other physiologically acceptable auxiliaries, for

example auxiliaries for adapting tonicity to the tonicity of blood or tissue, for example non-reducing sugars, sugar alcohols such as mannitol, sorbitol, xylitol or glycerin. Moreover, one or more amino acids
5 such as, for example, alanine, arginine, glycine, histidine or/and methionine, may be added to the formulation according to the invention to further increase the chemical stability. Methionine is preferred in this context. The methionine concentration
10 is preferably in the range of 0.1 to 4 mmol/l. A concentration of 2 mmol/l is especially preferred. Moreover, the composition may comprise thickeners for increasing the viscosity, for example for ophthalmological purposes. Examples of suitable thickeners are
15 ophthalmologically suitable polymers, for example Carbopol, methylcellulose, carboxymethylcellulose etc.

Moreover, the composition according to the invention may also comprise preservatives. For ophthalmological
20 purposes, for example, thiomersalate may be employed in an amount of 0.001 to 0.004% (weight/volume).

The invention furthermore relates to pharmaceutical preparations which comprise a liquid interferon- β -
25 comprising formulation as described above. These pharmaceutical preparations are particularly suitable for oral, parenteral or ophthalmological application. The formulations preferably exist in unit doses of 1 to 25 MU IFN- β . The invention furthermore relates to a
30 process for the preparation of such pharmaceutical preparation, in which a formulation according to the invention and, if appropriate, other pharmaceutical formulation auxiliaries which are necessary are prepared and formulated as a suitable dosage form.

35

The formulation according to the invention can be stored in suitable, washed and sterilized glass vials (hydrolytic class 1) with pharmaceutically acceptable rubber stoppers.

Moreover, formulations according to the invention can also be packaged aseptically in ready-to-use syringes or else in carpules for use in self-injection systems, and employed thus. While this is not preferred, the aqueous solutions may be freeze-dried by addition of other auxiliaries known to the skilled worker, and, after reconstitution, are available in liquid form.

Using suitable preservatives, it is possible to prepare liquid multidose forms and eye-drop solutions and solutions for dropwise oral application.

The auxiliaries additionally required for preparing the relevant dosage forms are known to the skilled worker.

Finally, the invention relates to a process for improving the shelf life of a liquid formulation which comprises human interferon- β as active ingredient and a buffer for setting a pH of 5 to 8, preferably of above 5.5 to 8, characterized in that a formulation without human serum albumin or/and with one or more amino acids is used. The improvement in shelf life encompasses improved long-term stability of the biological activity (in vitro), of the chemical integrity or/and of the physical integrity as indicated hereinabove.

The invention is furthermore illustrated by the examples which follow.

Examples

An interferon- β obtained from CHO cells was used in all the examples.

1. Long-term stability of liquid interferon- β formulations at 25°C

The following formulations were tested:

Formulation 1: 50 mmol/l sodium citrate pH 5.0

- Formulation 2: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 15 mg/ml human serum albumin, 2 mmol/l methionine, 50 mg/ml glycerin
- 5 Formulation 3: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 50 mg/ml glycerin, 2 mmol/l methionine
- Formulation 4: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 2 mmol/l methionine
- 10 Formulation 5: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0
- Formulation 17: 70 mmol/l sodium citrate, 50 mmol/l sodium phosphate, 2 mmol/l methionine, pH 6.5
- 15

The formulations were diluted to a content of approx. 10 to 15 MU/ml (that is to say 10 to 15 × 10⁶ IU/ml.

- 20 With the exception of formulation 17 (see below), the formulations were stored at 25°C for the period indicated in hydrolytic class 1 glass vials (DIN 2R vials) which were sealed with commercially available chlorobuthyl rubber stoppers. The biological activity
- 25 (in vitro) was determined as described by Stewart, W.E. II (1981): The Interferon System (Second, enlarged edition) Springer-Verlag: Vienna, New York; Grossberg, S.E. et al. (1984) Assay of Interferons. In: Came, P.E., Carter W.A. (eds.) Interferons and their
- 30 Applications, Springer-Verlag: Berlin, Heidelberg, New York, Tokyo, pp. 23-43.

The results are shown in Tables 1 to 5. "% (ref.)" indicates the biological activity based on the

35 biological activity of a reference sample which had been stored at -20°C for the period indicated. "% (0mo)" is the percentage of biological activity based on the initial value at 0 months.

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Table 1 (Formulation 1):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.0	11.0	100	100
1	10.0	9.8	98	89
2	9.7	11.0	113	100
3	10.0	10.6	106	96
4	10.3	9.5	92	86
5	9.5	9.7	102	88
6	10.5	10.2	97	93

Table 2 (Formulation 2):

5

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	13.9	13.9	100	100
1	14.0	11.9	85	86
2	13.0	11.6	89	83
3	13.1	9.6	73	69
4	12.5	8.8	70	63
5	11.0	8.2	75	59
6	13.3	8.4	63	60

Table 3 (Formulation 3):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	12.5	12.5	100	100
1	9.4	10.0	106	80
2	8.3	11.5	139	92
3	7.8	11.8	151	94.4
4	6.8	10.3	151	82.4
5	6.6	11.2	170	89.6
6	7.8	13.4	172	107.2

Table 4 (Formulation 4):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.4	11.4	100	100
1	10.5	10.2	97	89
2	11.9	11.1	93	97
3	10.8	10.0	93	88
4	10.4	9.3	89	82
5	11.6	8.4	72	74
6	12.4	9.5	77	83

5 Table 5 (Formulation 5):

Months	Active ingredient content			
	MU/ml		Recovery (25°C)	
	-20°C	25°C	% (ref.)	% (0mo.)
0	11.3	11.3	100	100
1	11.0	9.7	88	86
2	11.7	10.1	86	89
3	11.1	10.2	92	90
4	11.3	10.2	90	90
5	12.0	9.2	77	81
6	11.0	9.7	88	86

10 It can be seen from the above tables that formulations which do not contain human serum albumin (Formulations 1, 3, 4, 5) surprisingly exhibit a better stability than a formulation which comprises human serum albumin (Formulation 2).

15 In Formulation 17 (see above), an interferon solution without human serum albumin was brought to an activity of 6 MU/0.5 ml under aseptic conditions. The colourless, clear solution was subsequently filter-sterilized, and 0.5-ml aliquots were filled into pre-sterilized disposable syringes and sealed. The ready-

to-use syringes were stored at 25°C and examined for clarity, pH and biological activity. The following results were obtained:

Storage in months	pH	Clarity[%]	MU/syringe		Recovery (25°C)	
			-20°C	25°C	% (ref.)	% (0mo.)
0	6.5	99.5	6.3	6.3	100	100
3	6.5	99.1	5.6	6.1	108	97

5

2. Long-term stability of liquid IFN- β formulations at 37°C

10 The following formulations in ready-to-use syringes were tested:

- Formulation 6: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0, 2 mmol/l methionine
- 15 Formulation 7: 50 mmol/l sodium citrate pH 5.0, 18 mg/ml glycerin, 2 mmol/l methionine
- Formulation 8: 50 mmol/l sodium citrate pH 5.0, 18 mg/ml glycerin, 15 mg/ml human serum albumin, 2 mmol/l methionine
- 20 Formulation 9: 50 mmol/l sodium citrate pH 6.0, 18 mg/ml glycerin, 2 mmol/l methionine
- Formulation 10: 50 mmol/l sodium citrate pH 6.5, 18 mg/ml glycerin, 2 mmol/l methionine

25 The formulations were tested in dosage strengths of 3 MU per 0.5 ml (dosage strength 3), 6 MU per 0.5 ml (dosage strength 6) and 12 MU per ml (dosage strength 12).

30 The results are shown in Table 6 which follows.

Table 6

Storage in months	Dosage strength 3					Dosage strength 6					Dosage strength 12				
	Formulation					Formulation					Formulation				
0	6	7	8	9	10	6	7	8	9	10	6	7	8	9	10
	100	100	100	100	100	100	100	100	100	100	100	100	100	100	100
1	71	80	61	74	69	72	85	63	86	84	87	88	71	76	84
2	51	82	33	74	85	61	81	43	80	76	69	88	48	77	81
3	44	76	23	63	65	48	64	36	73	69	66	72	35	80	81
4	33	51	16	61	61	46	65	26	84	-	-	64	24	78	79

The results of Table 6 show that, surprisingly, the formulations according to the invention without human serum albumin exhibit an improved stability at 37°C.

5 3. Chemical stability at 25°C

10 To examine the chemical stability of liquid formulations of IFN- β , 7 batches were formulated and stored at 25°C. After 3 and 6 months, the protein was characterized by means of Lys-C mapping and complete carbohydrate analysis. The formation of methionine sulphoxide and the desialylation was checked particularly carefully.

15 In addition to Formulation 10 (see above), the following formulations were tested:

20 Formulation 11: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate, 2 mmol/l methionine pH 7.0 to 7.2

20 Formulation 12: 50 mmol/l sodium citrate, 50 mmol/l sodium phosphate pH 7.0 to 7.2

25 Formulation 13: 50 mmol/l sodium citrate, 18 mg/ml glycerin, 2 mmol/l methionine, pH 5.0 to 5.2

25 Formulation 14: 50 mmol/l sodium citrate, 18 mg/ml glycerin, pH 5.0 to 5.2

30 Formulation 15: 50 mmol/l sodium citrate, 15 mg/ml human serum albumin (medical grade), 18 mg/ml glycerin, 2 mmol/l methionine, pH 5.0 to 5.2

35 Formulation 16: 50 mmol/l sodium citrate, 15 mg/ml human serum albumin (medical grade), 18 mg/ml glycerin, pH 5.0 to 5.2 (comparison)

In all batches, the IFN- β content was between 10 and 11 MU/ml.

Testing procedure

To carry out the analyses, the samples had to be concentrated. Moreover, the human serum albumin had to be removed in the case of batches 15 and 16. This is why the batches were passed over an anti- β chromatography column. The initial volume per batch was 32 ml. Batches 13 to 16 were neutralized prior to anti- β chromatography by addition of 2.1 ml of 0.4 mol/l Na_2HPO_4 and 2.1 ml of 0.4 mol/l Na_3PO_4 .

For the immunoadsorption of interferon- β on a monoclonal antibody against interferon- β (BO2 sepharose 6B, crosslinked by Celltech), a C10 chromatography column (Pharmacia) was packed with 5 ml of BO2 sepharose and washed 3 times with in each case 5-10 gel volumes of PBS, 0.1 mol/l sodium phosphate pH 2.0 and PBS/1 mol/l KCl at a linear flow rate of 1.0 cm/min.

Approximately 32 ml of the interferon/HSA-containing solution was applied at a linear flow rate of 0.5 cm/min.

Washing was effected with 10 gel volumes of PBS/1 mol/l KCl with a linear flow rate of 1 cm/min until the OD had dropped to baseline. Elution was done with approximately 1-2 gel volumes of 0.1 mol/l sodium phosphate pH 2.0 at a linear flow rate of 1 cm/min. Interferon- β is obtained as single peak in high purity. This eluate is suitable for the subsequent protein characterization.

Analytical procedure

1. Lys-C mapping

Using the Achromobacter (AP) enzyme endoproteinase Lys-C, interferon- β is cleaved under reducing conditions on

the C-terminal end of lysin to give 12 peptides.

50 μ l of eluate from the anti- β chromatography (12.5-50 μ g of interferon- β) were placed into an Eppendorf reaction vessel, and 5 μ l of 2 mol/l TRIS were added. Wako endoproteinase was added in an enzyme/substrate ratio of 1:10 (endoproteinase Lys-C solution in 50 mmol/l TRIS/HCl, pH 9.0). The solution was mixed and incubated for 2 hours at 30°C. Then, 5 μ l of 0.1 mol/l DTT were added to the batch.

The peptides were separated on a reversed-phase column (Vydac C18, 300 Å, 5 μ m, 2.1 mm) on an HPLC system HP 1090 M series with diode array detector at 214 nm, for which purpose a gradient of A: 0.1% (v/v) TFA and B: 0.1% (v/v) TFA/70% (v/v) acetonitrile was used. The peptides were numbered consecutively in the sequence of their retention times and are allocated to the following sequences:

SEQ. ID. No.	Peptide	Position	Sequence
1	AP1	109-115	EDFTRGK
2	AP2	100-105	TVLEEK
3	AP3	46-52	QLQQFQK
4	AP4 (ox)	116-123	LM (ox) SSLHLK
5	AP4	116-123	LMSSLHLK
6	AP6 (ox)	35-45	DRM (ox) NFDIPEEIK
7	AP5	124-134	RYYGRILHYLK
8	AP6	34-45	DRMNFDIPEEIK
9	AP7	20-33	LLWQLNGRLEYCLK
10	AP8 (ox)	1-19	M (ox) SYNLLGFLQRSSNFQCQK
11	AP8	1-19	MSYNLLGFLQRSSNFQCQK
12	AP9	137-166	EYSHCAWTIVRVEILRNFYFINRLTG YLRN
13	AP10 (ox)	53-99	EDAALTIYEM (ox) LQNIFAIFRQDS SSTGWNETIVENLLANVYHQINHLK
14	AP10	53-99	EDAALTIYEMLQNIFAIFRQDSSSTG WNETIVENLLANVYHQINHLK

References:

Utsumi et al. (1989). Characterization of four different mammalian-cell-derived recombinant human interferon- β 1. Eur. J. Biochem. 181, 545-553.

- 5 Utsumi et al. (1988): Structural characterization of fibroblast human interferon- β 1. J. Interferon Res. 8, 375-384

Allen, G. (1981): Laboratory techniques in biochemistry and molecular biology. Sequencing of proteins and peptides. Elsevier Verlag.

- 10 Castagnola et al. (1988): HPLC in Protein sequence determinations. J. Chromatography 440, 213-251.

15 In the peptides marked (ox), the amino acid methionine is in the form of methionine sulphoxide. The quantification is based on determining the proportion of the peak area of the oxidized peptide relative to the total area of intact peptide and oxidized peptide. The proportions of oxidized methionines are very low in
20 fresh interferon- β preparations. However, the proportion increases more or less drastically during storage, depending on the storage conditions (buffer, pH, temperature etc.). This change is undesired since it contributes to the instability of the interferon- β
25 molecule or can significantly affect the in-vivo properties.

The proportion of the oxidized peptides AP4(ox), AP6(ox), AP8(ox) and AP10(ox) is thus an important
30 criterion for assessing the chemical integrity of the interferon- β molecule in a liquid formulation.

2. Carbohydrate determination

- 35 In the first step, the oligosaccharides were separated from the polypeptide and demineralized.

Approximately 0.7 ml of the eluate of the anti- β

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chromatography were dialysed for 16-20 hours at room temperature in a dialysis tubing (diameter 6 mm, Sigma No. D-9277) against 500 ml of dialysis buffer (0.05 mol/l sodium phosphate, 0.10 mol/l NaCl, pH 7.25), with gentle stirring. Then, the tubing was cut open at one end and the contents squeezed into a Eppendorf reaction vessel. After dialysis, the sample volume was 1 ml.

20 µl of Tween 20 (10% strength) and 15 µl of N-glycosidase F solution (Boehringer Mannheim) were pipetted to the dialysed sample. This mixture was incubated for 24 hours at 37°C. After the incubation had ended, the mixture was centrifuged for 10 minutes at 10,000 rpm, filtered through a 0.45 µm filter and subsequently chromatographed and fractioned over a desalting column (HR 10/10 Pharmacia No. 17-0591-01) with an isocratic gradient (eluent A: distilled water) at a flow rate of 1.0 ml/min. The free oligosaccharides were detected at 206 nm.

In the second step, the oligosaccharides which had been liberated were separated by an ion exchanger as a function of the number of the sialic acid residues.

The oligosaccharides contained in the eluate of the desalted column, approx. 2 ml, were bound to an anion exchanger (Mono Q HR 5/5, Pharmacia No. 17-0546-01). The asialo forms are in the eluate. With the aid of a shallow NaCl gradient, monosialo, disialo and trisialo forms eluted distinctly separately one after the other in the sequence indicated.

Eluent A: Milli-Q water
Eluent B: 0.10 mol/l NaCl

Gradient

0 min	100% A	0% B
5 min	100% A	0% B
25 min	33% A	67% B
26 min	100% A	0% B

Flow rate: 0.75 ml/min

Chromatography time: 26 min (with regeneration 36 min)

5 Detection: UV 206 nm

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10 The individual oligosaccharide fractions were detected by means of a UV detector at 206 nm. The quantitative calculation was done by integrating the areas of the individual peaks.

15 The oligosaccharide fractions monosialo, disialo and trisialo were subsequently passed over a desalting column as described above.

In the third step, the charged oligosaccharides are converted into neutral oligosaccharides by hydrolytically eliminating the terminal sialic acid residues under acidic pH conditions.

20 To this end, approx. 15 µl of each oligosaccharide fraction plus 15 µl of Milli Q water were placed into a micro-test tube, and 30 µl of 10 mmol/l H₂SO₄ were added. The mixture was then heated for 90 minutes at
25 80°C.

30 Then, the batch was centrifuged for 1 minute at 5000 rpm and pipetted into a minivial. The carbohydrates, which are now neutral, are bound at alkaline pH to weak anions and on an anion-exchanger column (CarboPac PA1 (4x250 mm) P/N 35391, Dionex). Elution is done with a gradient of

Eluent A: NaOH 0.16 mol/l

Eluent B: NaOH 0.16 mol/l sodium acetate 0.10 mol/l

Eluent C: NaOH 0.16 mol/l sodium acetate 0.75 mol/l

Gradient:

5

0 min	95% A	5% B	0% C
2.0 min	95% A	5% B	0% C
3.0 min	85% A	15% B	0% C
4.0 min	85% A	15% B	0% C
28.0 min	37% A	63% B	0% C
28.1 min	90% A	0% B	10% C
45.0 min	20% A	0% B	80% C
45.1 min	95% A	5% B	0% C
50.0 min	95% A	5% B	0% C

Flow rate: 1.0 ml/min

Chromatography time: 50 min

Detection: PAD

10

PAD (pulsed amperometric detection) was used to determine the oligosaccharides. The oligosaccharide molecule is electrochemically oxidized, and the current thus formed measured. PAD is distinguished by a high sensitivity, so that a detection in the ng range presents no difficulty. The output signal in the detector (in mV) is directly proportional to the amount of carbohydrate. Quantification is done by integrating the peak areas.

15

20

Between the deglycosylation and the analysis, the samples were subjected to intermediate storage at -20°C.

25 References:

Townsend (1988): High-performance anion-exchange chromatography of oligosaccharides. Analytical Biochemistry 174, 459-470.

Results

1. Lys-C mapping

- 5 The Lys-C mapping of batches 11 to 16 showed no difference to the initial value with regard to retention time and qualitative determination of the peptides.
- 10 The determination of the methionine sulfoxide content during liquid storage revealed the results shown in Tables 7 (3 months' storage) and 8 (6 months' storage).

Table 7

15

Name	AP4ox content	AP6ox content	AP8ox content	AP10ox content
to value	< 5%	7.6%	n.d.	n.d.
Formulation 11	7.9%	10.5%	n.d.	n.d.
Formulation 12	< 5%	11.6%	n.d.	n.d.
Formulation 13	< 5%	7.3%	n.d.	n.d.
Formulation 14	< 5%	9.4%	n.d.	n.d.
Formulation 15	< 5%	8.6%	n.d.	n.d.
Formulation 16	< 5%	10.8%	n.d.	n.d.

(n.d. = not detectable)

Table 8

Name	AP4ox content	AP6ox content	AP8ox content	AP10ox content
to value	< 5%	7.6%	n.d.	n.d.
Formulation 10	7.6%	8.9%	n.d.	n.d.
Formulation 11	7.7%	9.5%	n.d.	n.d.
Formulation 12	12.0%	13.7%	n.d.	n.d.
Formulation 13	7.4%	8.7%	n.d.	n.d.
Formulation 14	13.7%	15.7%	n.d.	n.d.
Formulation 15	7.4%	7.9%	n.d.	n.d.
Formulation 16	18.0%	17.6%	n.d.	n.d.

Table 7 reveals that the methionine-containing batches 13 and 15 show a lower methionine sulphoxide content upon three months' storage in comparison with methionine-free batches. After storage for six months, the affect of the added methionine in batches 11, 13 and 15 is more pronounced. Only a very small increase in the methionine sulphoxide content can be detected in these batches. In the methionine-free batches, the methionine sulphoxide content increases slightly more, but the total of all oxidized methionine contents amounts to less than 10% of the total methionine content.

2. Carbohydrate determination

The results of the carbohydrate determination after storage for three or 6 months are shown in Tables 9a, 9b, 10a, 10b, 11a and 11b.

Interferon- β -1a has a carbohydrate structure on its amino acid chain which is composed of a defined sequence of monosaccharides. Depending on the type of branching, these structures are termed biantennary (2 arms), triantennary (3 arms) and tetraantennary (4 arms).

The carbohydrate structure is composed of the monosaccharides mannose, fucose, N-acetylglucosamine, galactose and sialic acid.

In this context, the sialic acid is special in several respects:

- It is the only monosaccharide with a charged group (carboxyl group).
- It always occurs at the terminus of the carbohydrate chain.
- It can be eliminated enzymatically or

hydrolytically considerably more readily than the remaining monosaccharides.

- While the structure of the neutral carbohydrate chain is highly constant, the sialic acid moiety varies greatly depending, inter alia, on the cell culture and the purification method of the interferon.

References:

- 10 Kagawa et al., J. Biol. Chem. 263 (1988), 17508-17515; EP-A-0 529 300.

The sialostatus (percentage of individual sialo structures) after three months' storage (Table 9a) or six months' storage (Table 9b) was investigated. A carbohydrate structure which does not contain a terminal sialic acid is termed asialo. A carbohydrate structure which contains a terminal sialic acid is termed monosialo. A carbohydrate structure which contains two terminal sialic acids is termed disialo. A carbohydrate structure which contains three terminal sialic acids is termed trisialo.

Furthermore, the antennarity (percentage of individual branching types) was determined after three months' storage (Table 10a) and after six months' storage (Table 10b). A carbohydrate structure with one branching and thus two terminal galactoses is termed biantennary. It can have zero to two terminal sialic acids. A carbohydrate structure with two branchings and thus three terminal galactoses is termed triantennary. It can have zero to three terminal sialic acids.

The degree of sialylation (percentage occupation of terminal galactose residues with sialic acid) after three months' storage (Table 11a) and six months' storage (Table 11b) was also investigated.

It can be seen from the results that storage at pH 5 causes a slight, but reproducible, desialylation. Storage at pH 7 has no effect on the degree of sialylation.

5

The afuco content specified in batches 15 and 16 is probably due to foreign proteins from the added human serum albumin, which were not quantitatively removed by anti- β chromatography.

10

As regards the antennarity, liquid storage has no measureable effect.

Table 9a

15

Name	Asialo	Monoasialo	Disialo	Trisialo
to value	< 3	13.4	73.4	12.1
Formulation 11	< 3	14.0	74.1	11.9
Formulation 12	< 3	12.6	74.9	11.6
Formulation 13	< 3	16.5	70.4	12.0
Formulation 14	< 3	16.6	71.1	11.1
Formulation 15	< 3	15.8	70.0	13.0
Formulation 16	< 3	15.1	72.0	11.9

Table 9b

Name	Asialo	Monoasialo	Disialo	Trisialo
to value	< 3	13.4	73.4	12.1
Formulation 10	< 3	13.9	70.2	15.3
Formulation 11	< 3	14.5	73.9	11.6
Formulation 12	< 3	14.0	72.4	13.6
Formulation 13	< 3	18.6	68.9	11.7
Formulation 14	< 3	19.0	69.4	10.7
Formulation 15	< 3	17.0	71.0	11.3
Formulation 16	< 3	16.1	71.5	12.4

Table 10a

Name	Biantennary	Triantennary 1 → 6	Triantennary + 1 repeat
to value	74.4	18.1	3.7
Formulation 11	72.9	18.7	3.7
Formulation 12	76.9	17.0	2.7
Formulation 13	74.7	18.0	3.1
Formulation 14	75.9	17.3	2.9
Formulation 15	76.2 (incl. 5% afuco)	18.0	3.3
Formulation 16	76.9 (incl. 5% afuco)	17.8	3.0

Table 10b

5

Name	Biantennary	Triantennary 1 → 6	Triantennary + 1 repeat
to value	74.4	18.1	3.7
Formulation 10	71.4	19.3	4.0
Formulation 11	73.0	18.7	3.3
Formulation 12	72.3	19.7	3.4
Formulation 13	72.4	19.2	3.4
Formulation 14	74.2	18.7	3.2
Formulation 15	73.0	18.7	2.8
Formulation 16	74.3 (incl. 4% afuco)	19.7	3.2

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Table 11a

Name	Degree of sialylation
to value	88.3
Formulation 11	87.0
Formulation 12	88.2
Formulation 13	85.8
Formulation 14	85.8
Formulation 15	86.6
Formulation 16	86.9

Table 11a

5

Name	Degree of sialylation
to value	88.3
Formulation 10	87.5
Formulation 11	86.6
Formulation 12	87.7
Formulation 13	84.1
Formulation 14	84.3
Formulation 15	85.7
Formulation 16	86.5

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT:

- (A) NAME: Dr.Rentschler Biotechnologie GmbH
- (B) STREET: Erwin-Rentschler-Str. 21
- (C) TOWN: Laupheim
- (E) COUNTRY: Germany
- (F) POSTCODE: D-88471

(ii) TITLE OF THE INVENTION: Liquid interferon- β formulations

(iii) NUMBER OF SEQUENCES: 14

(iv) COMPUTER-READABLE VERSION:

- (A) MEDIUM TYPE: Floppy disk
- (B) COMPUTER: IBM PC compatible
- (C) OPERATING SYSTEM: PC-DOS/MS-DOS
- (D) SOFTWARE: Patent In Release #1.0, Version #1.30 (EPA)

(2) INFORMATION FOR SEQ ID NO: 1:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 7 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 109-115

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 1:

Glu Asp Phe Thr Arg Gly Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 2:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 6 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

5 (B) MAP POSITION: 100-105

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 2:

Thr Val Leu Glu Glu Lys
1 5

10

(2) INFORMATION FOR SEQ ID NO: 3:

(i) SEQUENCE CHARACTERISTICS:

15 (A) LENGTH: 7 aminoacids
(B) TYPE: aminoacid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

20

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 46-52

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 3:

25

Gln Leu Gln Gln Phe Gln Lys
1 5

(2) INFORMATION FOR SEQ ID NO: 4:

30 (i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 8 aminoacids
(B) TYPE: aminoacid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

35

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 116-123

40

(ix) FEATURE:

(A) NAME/KEY: Modified-site
(B) POSITION: 2
(C) OTHER INFORMATION: /product= "Xaa =
45 Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 4:

Leu Xaa Ser Ser Leu His Leu Lys
1 5

5 (2) INFORMATION FOR SEQ ID NO: 5:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 8 aminoacids
- (B) TYPE: aminoacid
- 10 (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

15 (viii) POSITION IN THE GENOME:

(B) MAP POSITION: 116-123

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 5:

Leu Met Ser Ser Leu His Leu Lys
1 5

20

(2) INFORMATION FOR SEQ ID NO: 6:

(i) SEQUENCE CHARACTERISTICS:

- 25 (A) LENGTH: 12 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

30 (ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 34-45

35 (ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) POSITION: 3
- (C) OTHER INFORMATION:/product= "Xaa =
Met(oxidized)"

40

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 6:

Asp Arg Xaa Asn Phe Asp Ile Pro Glu Glu Ile Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 7:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 11 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 124-134

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 7:

Arg Tyr Tyr Gly Arg Ile Leu His Tyr Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 8:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 12 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 34-45

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 8:

Asp Arg Met Asn Phe Asp Ile Pro Glu Glu Ile Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 9:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 14 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 20-33

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 9:

Leu Leu Trp Gln Leu Asn Gly Arg Leu Glu Tyr Cys Leu Lys
1 5 10

(2) INFORMATION FOR SEQ ID NO: 10:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 aminoacids

(B) TYPE: aminoacid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 1-19

(ix) FEATURE:

(A) NAME/KEY: Modified-site

(B) POSITION: 1

(C) OTHER INFORMATION:/product= "Xaa =
Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 10:

Xaa Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15
Cys Gln Lys

(2) INFORMATION FOR SEQ ID NO: 11:

(i) SEQUENCE CHARACTERISTICS:

(A) LENGTH: 19 aminoacids

(B) TYPE: aminoacid

(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 1-19

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 11:

Met Ser Tyr Asn Leu Leu Gly Phe Leu Gln Arg Ser Ser Asn Phe Gln
1 5 10 15
Cys Gln Lys

(2) INFORMATION FOR SEQ ID NO: 12:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 30 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 137-166

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 12:

Glu Tyr Ser His Cys Ala Trp Thr Ile Val Arg Val Glu Ile Leu Arg
 1 5 10 15
 Asn Phe Tyr Phe Ile Asn Arg Leu Thr Gly Tyr Leu Arg Asn
 20 25 30

(2) INFORMATION FOR SEQ ID NO: 13:

(i) SEQUENCE CHARACTERISTICS:

- (A) LENGTH: 47 aminoacids
- (B) TYPE: aminoacid
- (C) STRANDEDNESS: single
- (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

- (B) MAP POSITION: 53-99

(ix) FEATURE:

- (A) NAME/KEY: Modified-site
- (B) POSITION: 10
- (C) OTHER INFORMATION: /product= "Xaa = Met(oxidized)"

(xi) SEQUENCE DESCRIPTION: SEQ ID NO: 13:

Glu Asp Ala Ala Leu Thr Ile Tyr Glu Xaa Leu Gln Asn Ile Phe Ala
 1 5 10 15
 Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val
 20 25 30
 Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys
 35 40 45

(2) INFORMATION FOR SEQ ID NO: 14:

(i) SEQUENCE CHARACTERISTICS:

- 5 (A) LENGTH: 47 aminoacids
(B) TYPE: aminoacid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

10 (ii) MOLECULE TYPE: peptide

(viii) POSITION IN THE GENOME:

(B) MAP POSITION: 53-99

15 (xi) SEQUENCE DESCRIPTION: SEQ ID NO: 14:

Glu Asp Ala Ala Leu Thr Ile Tyr Glu Met Leu Gln Asn Ile Phe Ala
1 5 10 15
Ile Phe Arg Gln Asp Ser Ser Ser Thr Gly Trp Asn Glu Thr Ile Val
20 25 30
Glu Asn Leu Leu Ala Asn Val Tyr His Gln Ile Asn His Leu Lys
35 40 45

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Patent Claims

1. Liquid formulation which comprises human
interferon- β as active ingredient in a
concentration of up to 25 MU/ml and a buffer for
setting a pH of 5 to 8, is free from human serum
albumin and shows a long-term stability of the
biological activity (in vitro) of at least 80% of
the initial activity after storage for 3 months at
25°C.
2. Liquid formulation which comprises human
interferon- β as active ingredient and a buffer for
setting a pH of 6 to 7.2, is free from human serum
albumin and shows a long-term stability of the
biological activity (in vitro) of at least 80% of
the initial activity after storage for 3 months at
25°C.
3. Liquid formulation which comprises human
interferon- β as active ingredient, a buffer for
setting a pH of 5 to 8, and one or more amino
acids and shows a long-term stability of the
biological activity (in vitro) of at least 80% of
the initial activity after storage for 3 months at
25°C.
4. Formulation according to Claim 1,
characterized in that
it comprises a glycosylated interferon- β .
5. Formulation according to Claim 2,
characterized in that
the interferon- β originates from CHO cells.
6. Formulation according to any of Claims 1 to 5,
characterized in that

it comprises the buffer in a concentration of 10 mmol/l to 1 mol/l.

- 5 7. Formulation according to any of Claims 1 to 6,
characterized in that
it comprises a buffer selected from the group consisting of phosphate, citrate and acetate buffers and mixtures of these.
- 10 8. Formulation according to Claim 7,
characterized in that
it comprises a phosphate/citrate buffer.
- 15 9. Formulation according to any of Claims 1 and 3 to 8,
characterized in that
it has a pH between 6 and 7.2.
- 20 10. Formulation according to Claim 3,
characterized in that
it is free from human serum albumin.
- 25 11. Formulation according to any of Claims 1 to 10,
characterized in that,
apart from the active ingredient, it is free from human or animal polypeptides.
- 30 12. Formulation according to any of Claims 1 to 11,
characterized in that
it is free from surfactants.
- 35 13. Formulation according to any of Claims 1 to 12,
characterized in that
it exhibits a chemical integrity after storage for 6 months at 25°C.
14. Formulation according to any of Claims 1 to 13,
characterized in that

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it exhibits a physical integrity after storage for 6 months at 25°C.

- 5 15. Formulation according to any of Claims 1, 2 and 4 to 14,
characterized in that
it furthermore comprises one or more amino acids.
- 10 16. Formulation according to Claim 3 or 15,
characterized in that
it comprises methionine.
- 15 17. Formulation according to Claim 16,
characterized in that
the methionine is present in a concentration of 0.1 to 4 mmol/l.
- 20 18. Formulation according to any of Claims 1 to 17,
characterized in that
it furthermore comprises auxiliaries for adjusting the tonicity.
- 25 19. Formulation according to any of Claims 1 to 18,
characterized in that
it furthermore comprises thickeners for increasing the viscosity
- 30 20. Formulation according to any of Claims 1 to 19,
characterized in that
it furthermore comprises physiologically acceptable preservatives.
- 35 21. Pharmaceutical preparation,
characterized in that
it comprises a liquid formulation according to any of Claims 1 to 20.

22. Pharmaceutical preparation according to Claim 21 for oral, parenteral or ophthalmological administration.
- 5 23. Pharmaceutical preparation according to Claim 21 or 22 with unit doses of 1 to 25 MU.
- 10 24. Process for the preparation of a pharmaceutical preparation according to any of Claims 21 to 23, **characterized in that** a formulation according to any of Claims 1 to 20 and, if appropriate, other pharmaceutical formulation auxiliaries which are necessary is prepared and formulated as a suitable dosage form.
- 15 25. Process for improving the shelf life of a liquid formulation which comprises human interferon- β as active ingredient and a buffer for setting a pH of 5 to 8, **characterized in that** a formulation without human serum albumin or/and with one or more amino acids is used.
- 20 26. Process according to Claim 25, **characterized in that** the improved shelf life encompasses improved long-term stability of the biological activity (in vitro), of the chemical integrity or/and of the physical integrity.
- 25

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of:

Michael TSCHÖPE et al

Serial No.: 09/508,510

Filed: March 23, 2000

For: LIQUID INTERFERON- β FORMULATIONS

NOTIFICATION OF CHANGE OF NAME AND ADDRESS

Commissioner of Patents
Washington, D.C. 20231

May 26, 2000

Sir:

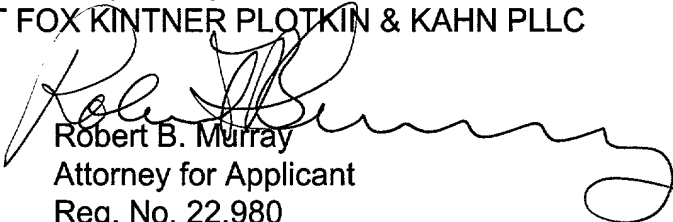
Kindly change the correspondence name and address for the above-identified application to the following:

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Telefax: (202) 638-4810

Should any fees be due with respect to this paper, please charge Counsel's Deposit

Account No. 01-2300.

Respectfully submitted,
ARENT FOX KINTNER PLOTKIN & KAHN PLLC


Robert B. Murray
Attorney for Applicant
Reg. No. 22,980

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RBM/cb

003250 "07530560

N, M, M & O Docket No. _____

NIKAIDO, MARMELESTEIN, MURRAY & ORAM

Declaration For U.S. Patent Application

As a below named inventor, I hereby declare that:

My residence, post office address and citizenship are as stated below my name.

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled
(Insert Title) Liquid interferon- β formulations

the specification of which

(Check one
of blocks
1, 2 or 3.
See note A
on back of
this page)

1. ☐ is attached hereto.
2. ☒ was filed on Sept. 23, 1998 as
International PCT Application Serial No. PCT/EP 98/06065
and was amended on Oct. 06, 1999 and Dec. 20, 1999
(if applicable)
3. ☐ was filed on _____ as
U.S. Application Serial No. _____
and was amended on _____
(if applicable)

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claim(s), as amended by any amendment referred to above.

I acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, §1.56(a).

I hereby claim foreign priority benefits under Title 35, United States Code, §119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application for which priority is claimed:

(List prior foreign applications. See note B on back of this page)	<u>97 116 562.6</u> (Number)	<u>EP</u> (Country)	<u>Sep. 23, 1997</u> (Day/Month/Year Filed)	Priority Claimed <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No
	_____ (Number)	_____ (Country)	_____ (Day/Month/Year Filed)	<input type="checkbox"/> Yes <input type="checkbox"/> No

(See Note C on back
of this page)

☐ See attached list for additional prior foreign applications

I hereby claim the benefit under Title 35, United States Code, §120 of any United States application(s) or PCT International application(s) designating the United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in the manner provided by the first paragraph of Title 35, United States Code, §112, I acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, §1.56(a) which occurred between the filing date of the prior application and the national or PCT International filing date of this application:

(List prior U.S. Applications or PCT International applications designating the U.S.)	_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status) (patented, pending, abandoned)
	_____ (Application Serial No.)	_____ (Filing Date)	_____ (Status) (patented, pending, abandoned)

And I hereby appoint as principal attorneys David T. Nikaido, Reg. No. 22,663; Charles M. Marmelstein, Reg. No. 25,895; George E. Oram, Jr., Reg. No. 27,931; Robert B. Murray, Reg. No. 22,980; Martin S. Postman, Reg. No. 18,570; E. Marcie Emas, Reg. No. 32,131; Michael G. Gilman, Reg. No. 19,114; Douglas H. Goldhush, Reg. No. 33,125; Kevin C. Brown, Reg. No. 32,402; Monica Chin Kitts, Reg. No. 36,105; Sharon N. Klesner, Reg. No. 36,335; and John R. Fuisz, Reg. No. 37,327.

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(202) 638-5000 Fax: (202) 638-4810

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

(See Note D
on back of
this page)

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Inventor's signature [Signature]

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Citizenship German

Post Office Address same as above

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4-0
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 Citizenship German
 Post Office Address same as above

Full name of fifth joint inventor, if any _____
 Inventor's signature _____ Date _____
 Residence _____
 Citizenship _____
 Post Office Address _____

Full name of sixth joint inventor, if any _____
 Inventor's signature _____ Date _____
 Residence _____
 Citizenship _____
 Post Office Address _____

Full name of seventh joint inventor, if any _____
 Inventor's signature _____ Date _____
 Residence _____
 Citizenship _____
 Post Office Address _____

Full name of eighth joint inventor, if any _____
 Inventor's signature _____ Date _____
 Residence _____
 Citizenship _____
 Post Office Address _____